

Comparative Morphology of the Glossopodia of Three North American *Isoetes* Ligules

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ABSTRACT.—One of the most distinctive features of the heterosporous lycopsids is the presence of a ligule. This structure, currently found only in *Isoetes* and *Selaginella*, is comprised of a basally embedded glossopodium and a free distal tongue. Previous studies on Indian species have demonstrated small variations in glossopodia suggesting the possibility that this structure could have taxonomic use. Serial cross, paradermal, and sagittal sections of glossopodia from three different North American species, representing three ploidy levels, were made. Three-dimensional digital rendering of the glossopodia provided comparative data for the three North America species and allowed comparisons with previously published descriptions. In general, the shape of the glossopodium is similar in all three North American species. There are several structural differences among them, such as the shape of the cornua, the length of the medimoles, and the angle of the glossopodium relative to the leaf axis.

The ligule is found only in the extant *Isoetes* and *Selaginella*. It is located on the adaxial surface of the leaf and is comprised of two major regions: a basal embedded glossopodium and a free visible portion, the tongue. The *Isoetes* glossopodium has a complex shape consisting of a transverse cellular band with globose lobes at each end, giving the overall appearance of a dumbbell. The tongue is parallel to the adaxial leaf surface and is deltate to triangular with auriculate bases. The tongue also consists of two sections, a multicellular central cushion and a thin peripheral margin.

The ligule is thought to have originated at least 408 million years ago during the Devonian (Goswami, 1976; Pigg, 1992, 2001) in lycopsids such as *Leclercqia complexa* Banks, Bonamo and Grierson (Grierson and Bonamo, 1979; Pigg, 2001). *Leclercqia complexa* is the earliest known ligulate lycopod and, as in all lycopsids, its ligule is positioned distal to the sporangium, but unlike any other ligulate lycopod, it is located 2/3 of the way up the leaf. *Leclercqia complexa*'s tongue tapers quickly to a rounded tip and averages 1.95 mm long by 1.80 mm wide (Grierson and Bonamo, 1979). Due to the type of fossil preservation, it is impossible to determine if *L. complexa*'s ligule possessed a glossopodium. Morphologists had long held that heterospory and ligules were correlated features in the lycopod line. With the discovery of *L. complexa* however, this assertion was proven untrue. *Leclercqia complexa* illustrates that the “origin of heterospory and the ligulate condition were not linked” because *L. complexa* is homosporous (Grierson and Bonamo, 1979; Pigg, 2001).

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Isoetaleans of the Carboniferous are represented by the lycopsids *Chaloneria cormosa* Pigg and Rothwell and *Chaloneria periodica* Pigg and Rothwell (= *Polysporia mirabilis* Newberry *sensu* DiMichele; Pigg and Rothwell, 1983). Anatomical characters are known for both species and it is evident that *C. cormosa* and *C. periodica* had corm-like axes and were anatomically similar. Their leaves possessed small ligules that lacked glossopodia (Pigg and Rothwell, 1983; Pigg, 1992; Retallack, 1997; Pigg, 2001).

By the Triassic, several morphological features characteristic of modern-day *Isoetes* had evolved. These include: monolete microspores, sunken sporangia, velum, labium, and elaborate ligules with glossopodia (Pigg, 2001). However, no single Triassic plant possessed all of these features (Pigg, 2001). Morphologically complex glossopodia were well developed by the mid-Triassic, as seen in *Takhtajanodoxa mirabilis* Snigirevskaya and *Isoetes beestonii* Retallack (Pigg, 1992; Retallack, 1997; Pigg, 2001). *Takhtajanodoxa mirabilis* had transfusion tissue between the glossopodium and the vascular bundle and possessed an intricate ligule with an anchor-like glossopodium that is similar to those of modern day *Isoetes* (Pigg, 1992). *Isoetes beestonii* is considered to be the earliest *Isoetes* (Retallack, 1997). *Isoetes beestonii* bore sporangia at their leaf bases and contained ligules with sunken glossopodia (Retallack, 1997). Pigg suggested that plants similar to modern-day *Isoetes*, such as *Isoetites rolandii* Ash and Pigg, emerged during the Jurassic (Pigg, 2001). Not only did *I. rolandii* have the general appearance and growth form of extant *Isoetes*, it was also completely fertile, producing only sporophylls at maturity as in modern *Isoetes* (Pigg, 2001). Pigg (1992) stated that such fossils document an evolutionary trend of increasing complexity in the glossopodial region of the Isoetalean ligule. By this, she was alluding to how the glossopodium has changed from the Carboniferous ligules that lacked glossopodia, through ligules with simple glossopodia, to extant ligules comprised of large glossopodia with intricate cornua (distal lobes on the glossopodium).

Ligules of *Isoetes* develop precociously on young leaves, being initiated as primordia just peripheral to the stem apex (Smith, 1900a; Sporne, 1966). The ligule arises from a large, single epidermal cell located on the adaxial surface of a leaf primordium when it is approximately five to seven cells tall (Smith, 1900a; Bhambie, 1963; Sporne 1966; Sharma and Singh, 1984). The ligule initial undergoes a paradermal division to form two daughter cells (Smith, 1900a; Bhambie, 1963). The inner cell develops into the glossopodium (Bhambie, 1963) and the outer daughter cell continues to divide, giving rise to a vertical file of three cells. These cells undergo repeated transverse and longitudinal divisions, forming a cellular plate oriented parallel to the epidermis (Bhambie, 1963; Sharma and Singh, 1984). This plate of cells is the visible laminate tongue of the ligule (Smith, 1900a; La Motte, 1933; Bhambie, 1963). The tongue grows quickly and soon overtops the young leaf primordium (Smith, 1900a; Bhambie, 1963; Sporne 1966; Sharma and Singh, 1984). Up to this point, most of the growth of the ligule has been two dimensional, but the central region eventually divides paradermally to become

multiseriate (Smith, 1900a; Bhambie, 1963; Sharma and Bohra 2002). This thickening begins in the basal region of the tongue and proceeds acropetally, but never extends to the margins or apex (Smith, 1900a; Bhambie, 1963). As a result, a central cushion and peripheral margins are differentiated.

As the ligule matures, the original interior cell divides vertically to form two cells. These cells divide irregularly to form a small horizontal band-like mass of cells. On either side of the band, rapid cell divisions occur, resulting in acropetal and basipetal growth and the development of the two lateral lobes known as the cornua (Figs. 1A, B, E, F, 2A, B; Smith, 1900a; Bhambie, 1963).

Thus, at maturity the ligule consists of two major regions: the tongue, commonly referred to simply as the ligule, and the glossopodium (Bhambie, 1963). The tongue is usually triangular and, after reaching maturity, is partly or wholly deciduous. It consists of a central cushion (Figs. 1A–D) whose cells are similar in size and shape to those of the glossopodium and which contain large quantities of protein and highly developed Golgi bodies (Kristen *et al.*, 1982). Lateral to the cushion is the margin (Figs. 1E, F), a region of the tongue that is only 1–3 cells thick. Margin cells usually have well developed endoplasmic reticulum (ER), but the cellular components and the cells themselves degrade quickly once the ligule reaches full size (Smith, 1900a, 1900b; Bhambie, 1963; Goswami, 1976; Kristen *et al.*, 1982; Sharma and Singh, 1984).

The glossopodium remains embedded and in most species is surrounded by a layer of sheath cells, which may be uniseriate or multiseriate (Fig. 1A; Bhambie, 1963; Goswami, 1976). This sheath is composed of small isodiametric gland-like cells and is the contact/boundary layer between the ligule and the leaf. The glossopodium itself is composed of isodiametric, parenchymatous cells that are arranged in an irregular pattern and are larger than the sheath cells (Fig. 1A). The free portion of the tongue is connected to the glossopodium by an embedded region that we call the medimoles (L., *media* = middle, *moles* = shapeless mass; Figs. 1B, C).

The physiological significance of *Isoetes* ligules is unknown (Kristen *et al.*, 1982), but numerous hypotheses have been put forward. These hypotheses include physical protection of leaf primordia (Sharma and Bohra, 2002), desiccation protection for sporangia and young leaves (Bierhorst, 1971; Goswami, 1976; Sharma and Singh, 1984; Gifford and Foster, 1989), nutritive/transport functions (Goswami, 1976; Kristen and Biedermann, 1981; Sharma and Bohra, 2002), water retention (Bierhorst, 1971; Sharma and Singh, 1984), and movement of solutes (Bierhorst, 1971). Recently, Kristen *et al.* (1982) has suggested that the ligule may have antibacterial properties. This plethora of hypotheses, most of which are based on similar data sets provides us with little confidence in any single one, either because the ligule is involved in several functions or because we have not yet identified the correct hypothesis. In any event, experimental work will be necessary to clarify this issue.

Selaginella and *Isoetes* are ligulate, heterosporous, (Sharma and Singh, 1984; Gifford and Foster, 1989) and share numerous developmental and vegetative characters. For example, both have endosporic gametophytes (Bierhorst, 1971; Gifford and Foster, 1989), similar embryo orientation (La Motte, 1933), and

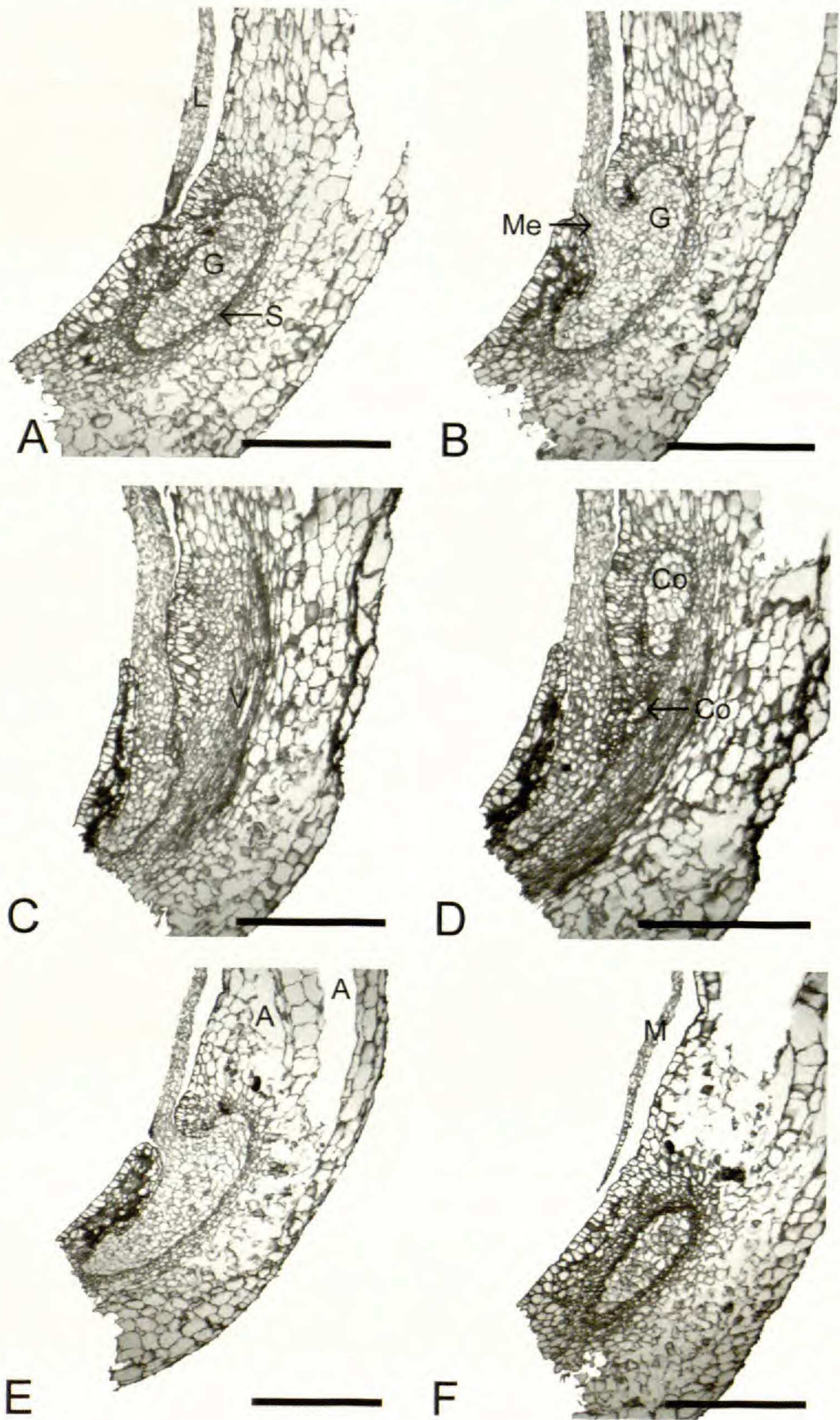


FIG. 1. Serial sagittal sections of *Isoetes virginica*. A. The glossopodium is bean shaped and not (in this sectional plane) connected to the tongue. B. The tongue is connected to the glossopodium by a short, slightly upwardly-angled medimoles. C. In medial section, the tongue, medimoles, and glossopodium are impossible to differentiate. D. There are two portions of the cornu lobe distal to

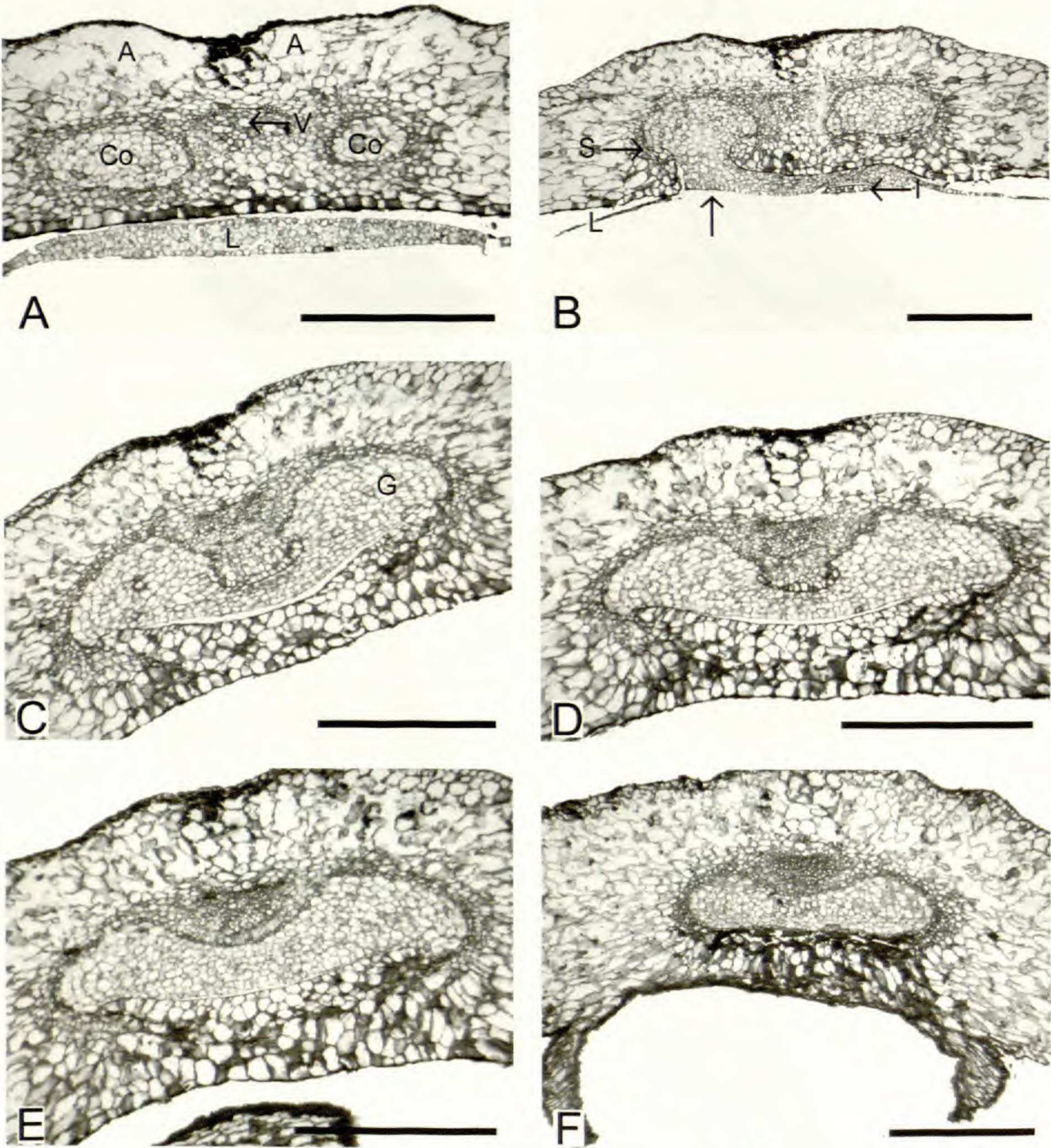


FIG. 2. Serial cross sections of *Isoetes virginica*. A. Uppermost section, the cornua are depressed-ovate in shape. B. The cushion is attached to the cornua by broad, radially short medimoles sections (arrow). C, D. The transverse band connects the cornua forming the glossopodium. E, F. The glossopodium is relatively simple and has reduced cornua. Scale bars = 500 μm. A = lacuna, Co = cornu, G = glossopodium, I = labium, L = tongue, S = sheath, V = vascular trace.

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the glossopodium. E. The glossopodium is at its most complex form, as in section B. F. The detached ligule margin and a simple conua lobe are evident. Scale bars = 500 μm. A = lacuna, Co = cornu, G = glossopodium, L = tongue, M = margin, Me = medimoles, S = sheath, V = vascular trace.

leaves that initiate from an assemblage of superficial cells (Smith, 1900b; Bhambie, 1963; Gifford and Foster, 1989). There are many differences as well. For instance, *Selaginella* embryos have suspensors, whereas *Isoetes* embryos lack them (Gifford and Foster, 1989); *Selaginella* sperm is biflagellate, whereas *Isoetes* sperm is multiflagellate (Bierhorst, 1971; Gifford and Foster, 1989); *Selaginella* leaves are dimorphic and lack foliar air chambers, whereas *Isoetes* leaves are monomorphic and contain four series of air chambers (Webster, 1992; Moran, 1995; Sharma and Bohra, 2002).

Ligule ontogeny is also dissimilar between *Selaginella* and *Isoetes*. In *Selaginella* the ligule develops from two rows of superficial cells (Smith, 1900a; Horner *et al.*, 1975; Gifford and Foster, 1989), whereas in *Isoetes* it originates from a single epidermal cell (Smith, 1900a; Bhambie, 1963; Sporne, 1966; Gifford and Foster, 1989). In both, the ligules are attached to the adaxial leaf surface distal to the sporangium, produce callose, achieve maturity before their corresponding leaves, and lack chlorophyll, starch, and intercellular spaces (Smith, 1900a; Bierhorst, 1971; Horner *et al.*, 1975; Jagels and Garner, 1979; Kristen and Biedermann, 1981; Webster, 1992). At maturity, the ligules of each consist of four sections. In *Selaginella* these are the sheath, glossopodium, bulbous base, and tip or neck. These are comparable to the four regions of the *Isoetes* ligule (Smith, 1900a, 1900b; Bhambie, 1963; Sigee, 1974; Horner *et al.*, 1975; Kristen *et al.*, 1982; Bilderback, 1987; Bilderback and Slone, 1987) but the individual parts differ in size and extent of development. *Selaginella* ligules are rarely if ever triangular and instead are shaped like a slightly curved, cupped hand. They also have a much simpler glossopodium (Smith, 1900a, b; Horner *et al.*, 1975; Gifford and Foster, 1989), lacking the broad cornua typical of *Isoetes*.

The ligules of *Selaginella* and *Isoetes* are quite similar at the ultrastructure level. Both contain dense cytoplasm, protein bodies, Golgi, ER, and mitochondria, although *Isoetes* appears to contain more Golgi and ER than does *Selaginella* (Paolillo, 1962; Sigee, 1974; Kristen and Biedermann, 1981; Kristen *et al.*, 1982; Bilderback and Slone, 1987). The presence of ER suggests significant amounts of protein synthesis (Kristen and Biedermann, 1981; Kristen *et al.*, 1982). This is further evidenced by temporary protein bodies within the ligule (Kristen *et al.*, 1982). These ligular protein bodies are identical to those found in the external mucilage (Kristen and Biedermann, 1981; Kristen *et al.*, 1982). The secreted mucilage from *Selaginella* and *Isoetes* ligules (Bhambie, 1963; Kristen *et al.*, 1982; Bilderback, 1987; Bilderback and Slone, 1987; Webster, 1992) consists of two major components: proteins and polysaccharides (Paolillo, 1962; Kristen *et al.*, 1982; Webster, 1992).

Despite these similarities, there are ultrastructural differences. Kristen *et al.* (1982) showed that the ligule cushion of *Isoetes lacustris* L. possess numerous protein bodies, has connections between Golgi and ER, and lacks cell wall ingrowths within the cushion. The ligule margins also showed well developed ER, Golgi, and mucilage. Kristen *et al.* (1982) argued these as evidence that the ligule is to be "considered a secretional organ". In contrast, the ligule base of *Selaginella kraussiana* (Kunze) A. Braun. lacks protein bodies, has no known Golgi-ER connections, and the ligule bases of *Selaginella pilifera* A. Braun and

Selaginella uncinata (Desv. ex Poir.) Spring contain cell wall ingrowths (Kristen *et al.* 1982). *Selaginella* ligule margins lack well developed ER, Golgi, and mucilage (Kristen *et al.* 1982). The lack of these structures, suggest that *Selaginella* ligules do not secrete mucilage. That hypothesis was supported by the studies of Sigee (1974), Bilderback (1987), and Webster (1992) who showed that some *Selaginella* species do not secrete mucilage.

Previous studies on the ligule of *Isoetes* have demonstrated variation in glossopodium shape among the Indian species *I. reticulata* Gena and Bhardwaja, *I. coromandelina* L.f., and *I. rajasthanensis* Gena and Bhardwaja. The cornua of the glossopodia have been reported as triangular, anchor-shaped, or globular (Figs. 3A–C; Sharma and Singh, 1984), suggesting that the glossopodium may have some taxonomic value. Sharma and Singh's work inspired the current research on glossopodium morphology of several North American *Isoetes*. Specifically, the current work asks if glossopodium shape is consistent among North American *Isoetes*, if the cornua of North American species are similar in shape to any of those described for the Indian *Isoetes* species, and if 3-D images derived from different sectioning planes can be used to faithfully reflect glossopodium morphology?

METHODS AND MATERIALS

Three specimens of *I. melanopoda* Gay and Durieu (2×), four of *I. virginica* N. Pfeiff. (4×), and five of *I. tennesseensis* Luebke and Budke (8×) were collected and fixed in FAA (Table 1). After fixation, the plants were moved into 70% ethanol for long-term storage. Basal portions of mature megasporophylls were removed, dehydrated in a TBA series, and embedded in Paraplast (Johansen, 1940). Serial cross, paradermal, and sagittal sections were prepared using a rotary microtome set at 10µm. Ribbons were mounted onto glass slides using egg albumen (Johansen, 1940) and stained with 0.2% toluidine blue O. Each section was magnified 37× with a Rayoscope slide projector and the glossopodium was traced onto paper. Angular orientations of the glossopodium and medimoles relative to the leaf axis were measured from these tracings. Nine sets of glossopodium tracings, three from each species, were scanned into a computer and aligned by hand using Adobe Photoshop (version 6.0) and Image Pro Plus (version 4.5). 3-D images of each were created using Voxblast (version 3.0) and were saved in Quick Time and AVI format. In the 3-D images, the tongues were erased, except in the three sagittal sections, to conserve computer memory and because they were not an integral part of this study. Photographs of thin sections were taken with a Nikon Coolpix 4500 digital camera mounted on an Olympus BH-2 microscope. All measurements were made directly from slides with the aid of an ocular micrometer. Glossopodia and cornua shapes were determined using Radford (1986) symmetric plane figures.

RESULTS

Isoetes virginica.—In the first peripheral sagittal section (Fig. 1A), the glossopodium is elongate and bean shaped; its orientation is parallel to the leaf

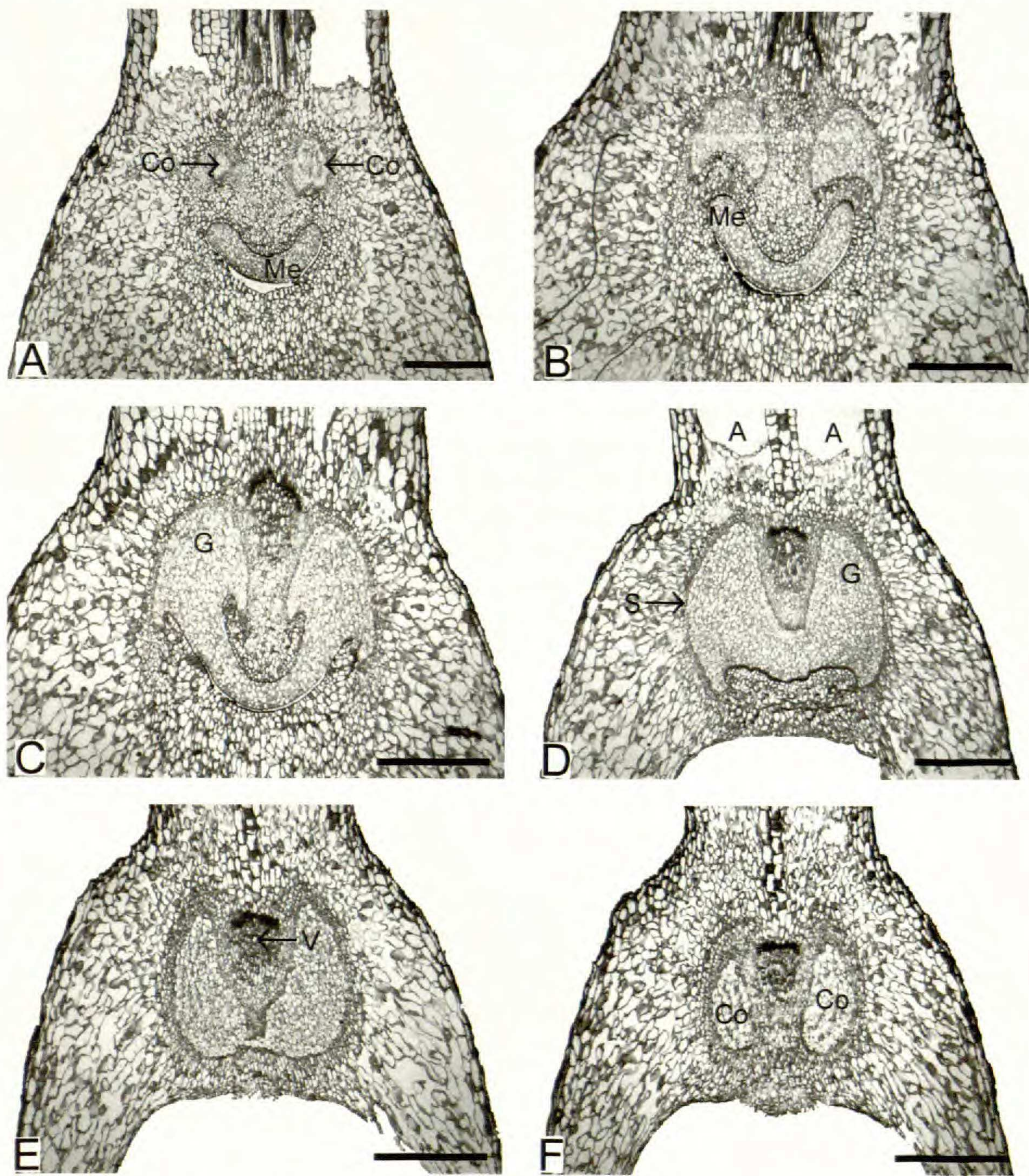


FIG. 3. Near paradermal longitudinal sections of *Isoetes virginica*, starting from the adaxial face and proceeding abaxially. A, B. The curved medimoles is in the foveola with two cornua lobes distal to it. C. The transverse band is continuous with the cornua lobes, note small protuberances on the lower portions of the cornua. D. The cornu lobes are more extensive and the transverse band is flatter and thicker. E. The glossopodium is reduced to a simpler form. F. The transverse band is gone, leaving only two cornu lobes. Scale bars = 500 μ m. A = lacuna, Co = cornu, G = glossopodium, Me = medimoles, S = sheath, V = vascular trace.

TABLE 1. Collection information for study material. N stands for the number of leaves sectioned for each species.

Species	Collection	N
<i>I. melanopoda</i> Gay & Durieu	New Salem Union County, North Carolina. Kerry Heafner 99011 (MU) 16-April-2002.	3
<i>I. virginica</i> N.E. Pfeiffer	Person County, North Carolina. Kerry Heafner 99015 (MU) 18-April-2002.	4
<i>I. tennesseensis</i> Luebke & Budke	Polk County, Tennessee. Jessica Budke et al. 3-04TN (MU) 15-July-2001.	5

axis. The ligule cushion is seen as detached from the rest of the leaf, but the extreme edge of the foveola (ligular pit) is noticeable between the base of the tongue and the center of the cornu (Fig 1A). More medially (Fig. 1B), the tongue is connected to the glossopodium by a short medimoles that is angled slightly upward and away from the cornu. In medial sagittal section (Fig. 1C), the cushion, medimoles, and glossopodium are similar in thickness forming a continuum and lacking differentiation. At this point, the entire glossopodium lies quite close to the adaxial leaf surface. In Fig. 1D, two portions of the cornu are evident just abaxial to the rest of the glossopodium. In sequentially more peripheral sections (Figs. 1E, F) the proximal cornu exhibits a form similar to that of the distal one (Fig. 1B). Lacunae are evident above the glossopodium in Fig. 1E. In the last section (Fig. 1F), the tongue margin is isolated and the peripheral portion of the cornu is visible.

In distal serial cross section (Fig. 2A), the ligule cushion is noticeably thick and tapers laterally into the margins. The cornua are depressed-ovate with flattened adaxial faces (Fig. 2A). At this point, the vascular trace is located between the cornua, and the two abaxial lacunae are evident. Slightly lower, a small portion of the labium is evident just adaxial to the leaf surface (Fig. 2B). At this point, one of the cornua lobes is attached to the cushion by means of two broad, but radially short portions of the medimoles (arrow in Fig. 2B) and small protuberances on the adaxial corners of the cornua are visible. In lower sections, the transverse band connecting the cornua thickens and the cornua lose their distinctness (Fig. 2C, D). The glossopodium base is simple and dumb-bell shaped (Figs. 2E and F).

In proximal, paradermal longitudinal sections of *I. virginica* the curved medimoles is shown within the foveola with two unattached cornua in a more distal position (Figs. 3A, B). Moving abaxially, the thin, broadly curved transverse band attaches to the cornua between small protuberances (Fig. 3C). Abaxially from this point the transverse band flattens and thickens as the cornua become more extensive (Fig. 3D). At this point, two lacunae are visible distal to the glossopodium and a noticeable sheath surrounds the glossopodium (Fig. 3D). In the next two figures, the transverse band is increasingly reduced and the cornu lobes again become more distinctive. The vascular trace extends

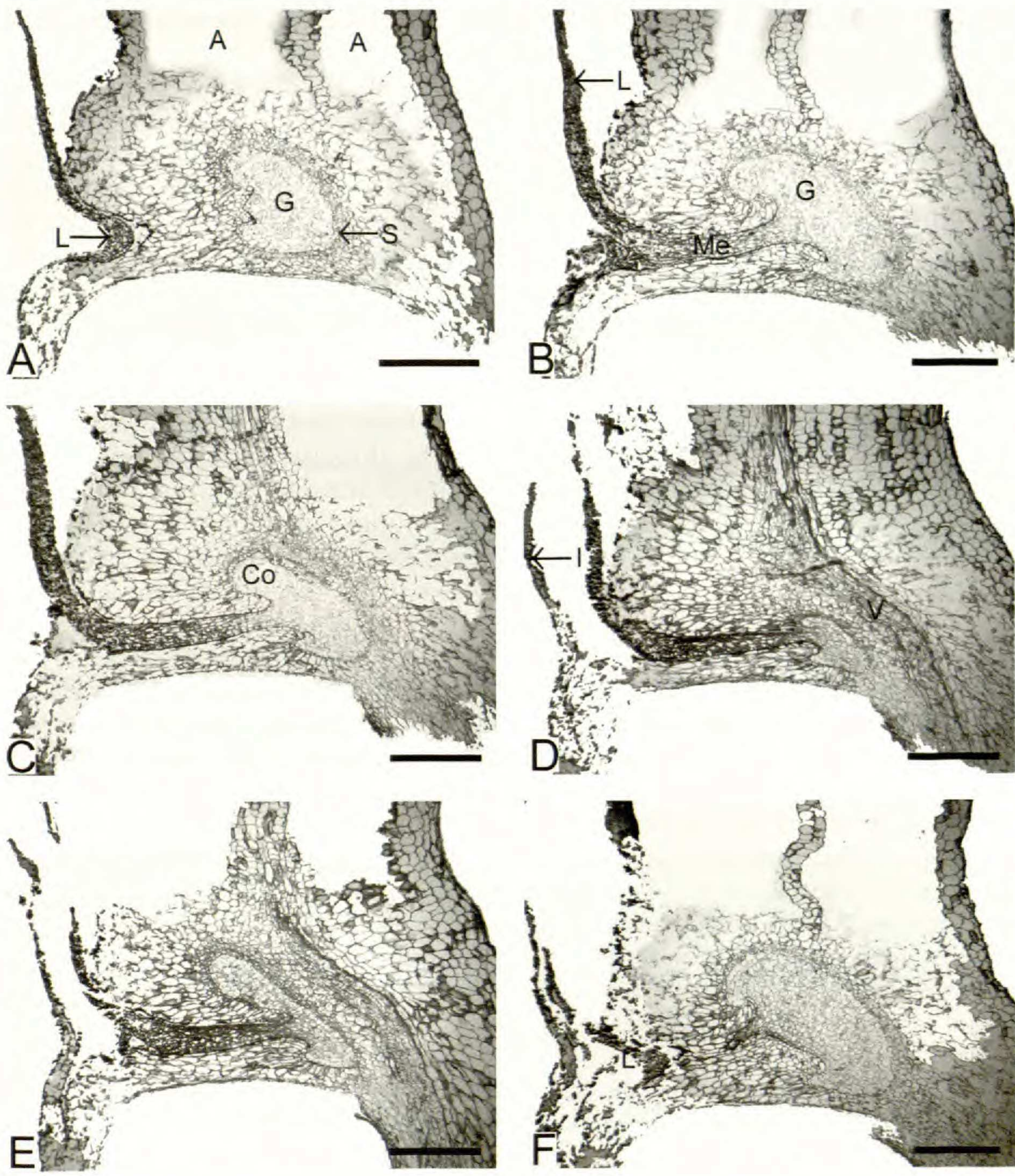


FIG. 4. Serial sagittal sections of *Isoetes tennesseensis*. A. The tongue is intruding into the foveola in front of the ovate glossopodium. B, C. The tongue is attached to the glossopodium by a long, horizontal medimoles. D. Medial section with a highly reduced glossopodium. E. The glossopodium is again at its larger, more complex form (as in sections B and C). F. The tongue is not connected to the glossopodium, but segments of it and the medimoles remain in the foveola. Scale bars = 500 μ m. A = lacuna, Co = cornu, G = glossopodium, I = labium, L = tongue, Me = medimoles, S = sheath, V = vascular trace.

between these lobes (Figs. 3E, F). In the last view (Fig. 3F), the transverse band is completely absent and the two cornu are fully distinct.

Isoetes tennesseensis.—In peripheral sagittal section of *I. tennesseensis* (Fig. 4A) the tongue is seen intruding into the lateral edge of the foveola. At this point, the glossopodium is somewhat ovate and is enveloped by a noticeable sheath. Two lacunae are evident above the glossopodium (Fig. 4A). More medially, the tongue is attached to the adaxial face of the glossopodium by a long medimoles (Fig. 4B). In medial (Fig. 4B) and near medial (Fig. 4C) sections of a cornu the glossopodium is at its most complex condition. At this point in *I. tennesseensis*, the cornua tilt adaxially at an acute angle. In absolute medial sections, the ovate glossopodium is diminutive and the vascular trace is visible abaxial to it (Fig. 4D). Fig. 4E is a section through the proximal complex segment of the glossopodium and has features similar to those of Fig. 4B. In Fig. 4F, the tongue is discontinuous with the glossopodium, but is still contained within the foveola. Also, a short peripheral portion of the medimoles can be seen projecting toward the ligule from the upper portion of the cornu.

In the most distal cross section of *I. tennesseensis* (Fig. 5A), a labium is visible in front of the ligule tongue. At this level, the cornua are separate, transversely elliptical, and are surrounded by a noticeable sheath (Fig. 5A). The vascular trace is positioned between the cornua and two lacunae are visible near the abaxial leaf surface (Fig. 5A). In Fig. 5B, the tongue (arrow) is encroaching the foveola, adaxial to one of the cornua. Protuberances are noticeable on either side of the concave adaxial surface of the cornua. Slightly lower, the tongue is connected to the adaxial surface of the cornua by two lateral portions of the medimoles (Fig. 5C). At this level, both cornua curve adaxially along their outer edges (Fig. 5C). In Fig. 5D, only the basal auricles of the ligule are visible and the glossopodium is connected centrally to the broad medimoles. A thin transverse band linking the curved cornua is evident (Fig. 5D). In successively lower sections, the labium has merged with the leaf and a simple elliptical pad of tissue represents the glossopodium (Figs. 5E, F). The fovea is noticeable in front of the glossopodium (Fig. 5F).

Isoetes melanopoda.—In the peripheral sagittal sections of *I. melanopoda* (Fig. 6A), the detached ligule cushion intrudes into the foveola. A small obovate patch of glossopodium is adjacent to the intruding tongue and two lacunae are located above the glossopodium (Fig. 6A). Closer to the center, a pronounced labium and extensive medimoles is evident. The medimoles connects centrally to the near vertically oriented glossopodium (Fig. 6B). The sheath is clearly evident at this level (Fig. 6B). In medial sections (Fig. 6C), the glossopodium is represented by the small, terete transverse band and the vascular trace curves around and abaxial to the glossopodium. Fig. 6D is a section through a more proximal segment of the glossopodium just at the edge of a cornu. It has features similar to those of Fig. 6B. More laterally, the ligule margin is visible intruding into the foveola, but at this point, is not attached to the medimoles (Fig. 6E). Fig. 6F is a peripheral section through the glossopodium; only the ligule margin is visible as is a small, elliptical patch of tissue representing the cornu.

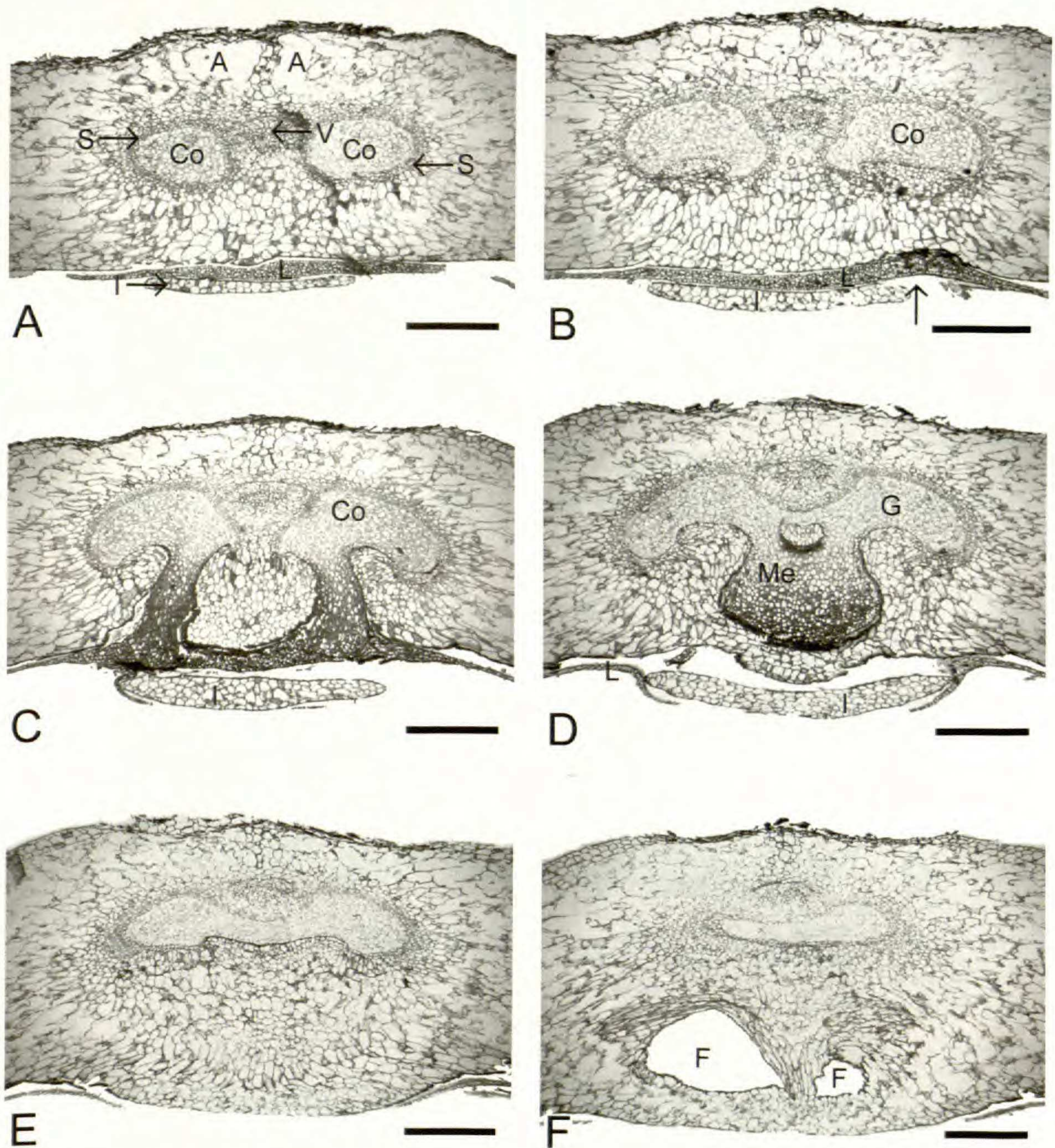
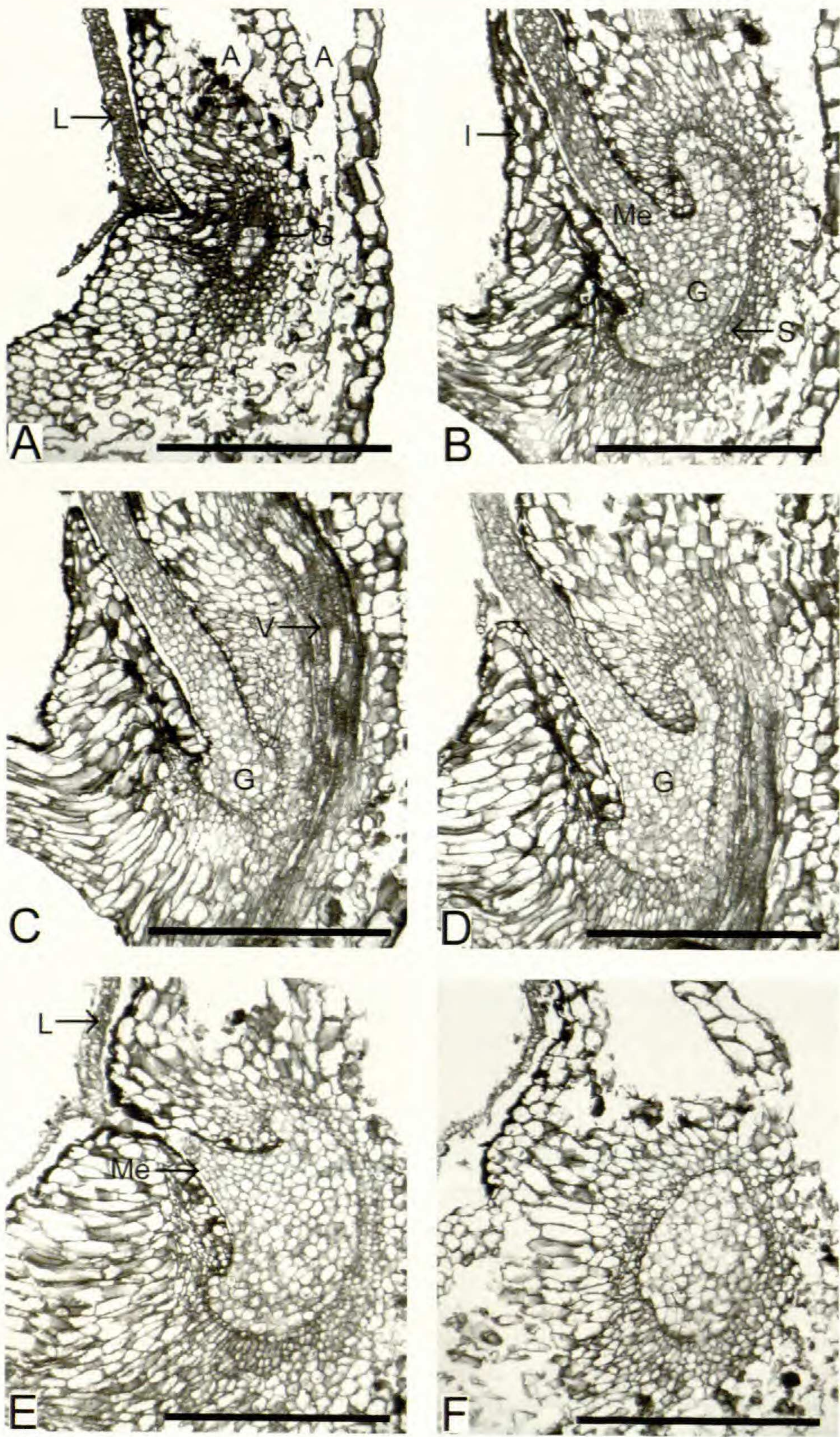


FIG. 5. Serial cross sections of *Isoetes tennesseensis*, proceeding from the top down. A. Section through the elliptical cornua lobes. B. The tongue encroaching into the foveola (see arrow). C. The tongue is connected to the cornua by two lateral portions of the medimoles. D. The glossopodium is attached to the broad medimoles. E. The labium has merged with the leaf and the glossopodium is reduced to a simple pad. F. The fovea is noticeable in front of the simple glossopodium. Scale bars = 500 μ m. A = lacuna, Co = cornu, F = fovea, G = glossopodium, I = labium, L = tongue, Me = medimoles, S = sheath, V = vascular trace.

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FIG. 6. Serial sagittal sections of *Isoetes melanopoda*. A. The tongue is intruding into the foveola. Adjacent to the tongue is a small obovate portion of one cornu. B. The tongue is connected to the glossopodium by an ascending medimoles. C. Medial section, the glossopodium (transverse bar) is



small and terete. D. Complex glossopodium architecture similar to that of section B. E. The tongue is disconnected from the glossopodium, but it and a segment of the medimoles is seen within the foveola. F. The ligule margin and a small elliptical patch of glossopodium are evident. Scale bars = 500 μ m. A = lacuna, G = glossopodium, I = labium, L = tongue, Me = medimoles, S = sheath, V = vascular trace.

TABLE 2. Differences in the glossopodia of three North American species of *Isoetes*.

	<i>I. tennesseensis</i>	<i>I. virginica</i>	<i>I. melanopoda</i>
Paradermal view of cornua	Stout triangular	Elliptic	Ovate
Sagittal view of cornua	Reniform	Thin, narrowly elliptic	Elliptic
Ligule attachment position on the glossopodium	Slightly below the center	High	Centrally
Medimoles size	Large: well developed	Stout: not extensive	Short: not extensive
Medimoles angular departure from the glossopodium	Parallel	Parallel	Ascending
Angle of the glossopodium compared to vertical leaf axis	Upper section leans acutely adaxially	Near parallel to the leaf axis	Upper section leans slightly abaxially
Maximum cornu height and glossopodium width (μm)	Height = 1120 Width = 1900	Height = 860 Width = 1200	Height = 540 Width = 820

In distal cross section (Fig. 7A), the cornua of *I. melanopoda* are depressed-ovate with a somewhat flattened adaxial face. A multiseriate sheath surrounds each cornu. The vascular trace is located between the cornua and two lacunae are evident. Moving basipetally, the tongue merges with the adaxial face of the cornua by means of the two (one shown in Fig. 7B) lateral edges of the medimoles. In successively lower sections the medimoles transitions into the transverse band, which connects the two cornua (Figs. 7C, D). Small cellular protuberances are located on the inside (Fig. 7C) and outside corners (Fig. 7D) of the cornua giving them a bulbous, angular appearance. At this level the glossopodium resembles a curved dumb-bell (Figs. 7D, E). In lower sections (Figs. 7E, F), the transverse band is thicker and the cornua are less distinct.

Using the cross sectional and sagittal sectional views, each North American species was measured to determine its maximum glossopodium width and cornu height (Table 2). *Isoetes tennesseensis* is the largest with a maximum width of 1900 μm and a cornu height of 1120 μm . *Isoetes virginica* has a maximum glossopodium width of 1200 μm and a cornu height of 860 μm . *Isoetes melanopoda* is the smallest of the three, with a glossopodium width of 820 μm and a cornua height of 540 μm .

The nine images of Fig. 8 are the starting images of nine movie reconstructions produced from serial cross, sagittal and paradermal sections. Based on these reconstructions it is clear that the ligules of all three North American species are similar in overall form. All the ligules have a well developed sheath, glossopodium, cushion, and margin. In each, the tongue is attached to the glossopodium adaxially via the medimoles. Each glossopodium is symmetrical and bilobed, has complex cornua, is proximal to the lacunae, and has the vascular trace passing between the cornua. Despite these similarities, there are several structural variations among the North American species.

In abaxial face view, the cornua of *I. virginica* are elliptic (Figs. 3D, 8E). In sagittal view, the glossopodium appears thin and narrowly elliptic, and the tongue attaches high on the adaxial face (Figs. 1E, 8F). In lateral and cross sectional views, it is evident that the medimoles is stout but not extensive

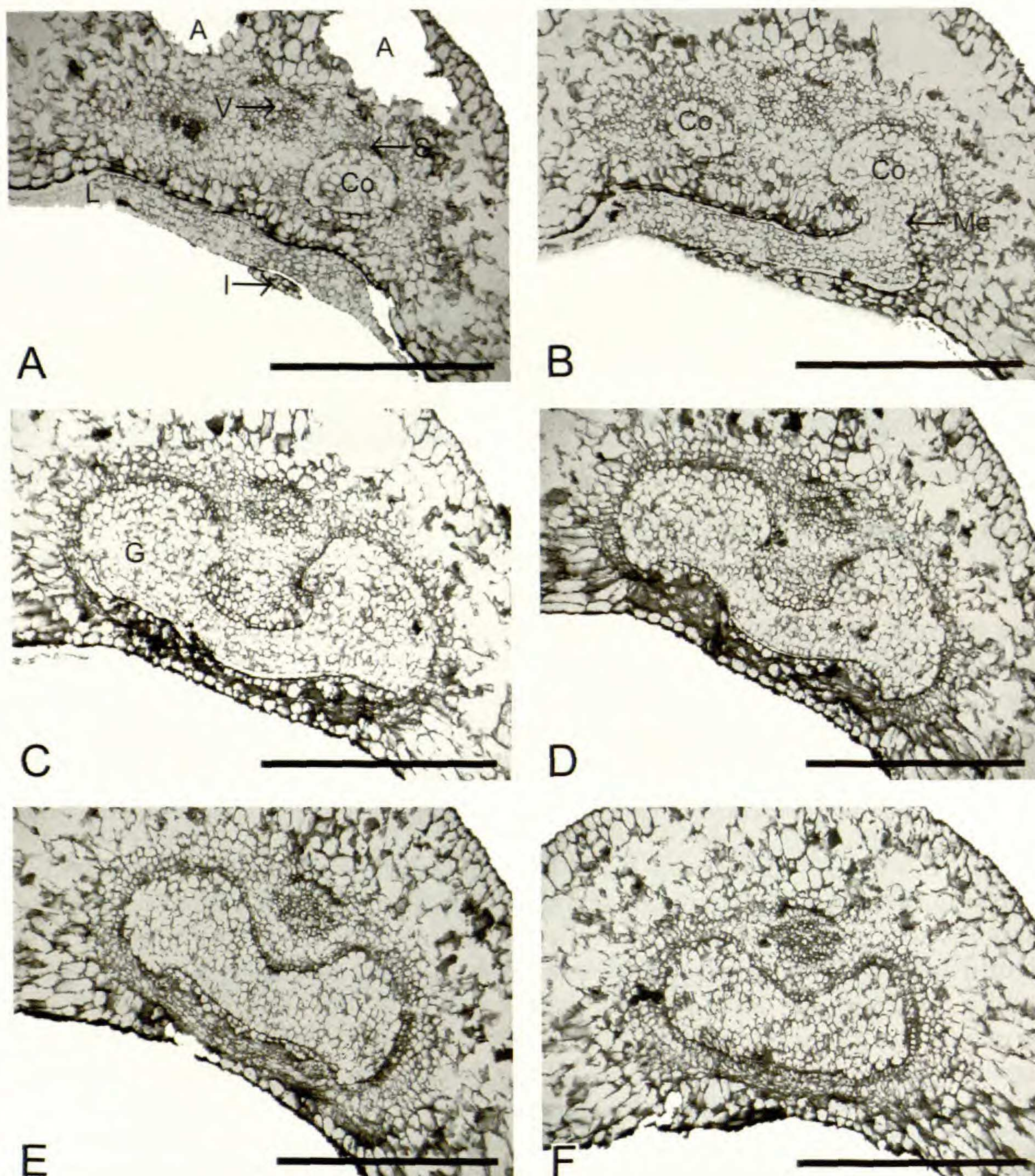


FIG. 7. Serial cross sections of *Isoetes melanopoda*, proceeding basipetally. A. The cornua are depressed ovate and the tongue is at the edge of the foveola. B. The tongue is connected to the cornua by two (one shown) edges of the short medimoles. C, D. The transverse band connects the two cornua; the glossopodium is dumb-bell shaped. E, F The cornua are less distinct, giving the glossopodium a simpler appearance. Scale bars = 500 μm . A = lacuna, Co = cornu, G = glossopodium, I = labium, L = tongue, Me = medimoles, S = sheath, V = vascular trace.

(Figs. 1B, 2B, 8D, 8F). The glossopodium axis is nearly parallel to the leaf axis (Figs. 1B, 8F).

In face view, the cornua of *I. tennesseensis* are stout-triangular and somewhat flattened ventrally (Fig. 8B). Viewed from the side the cornua appear reniform in

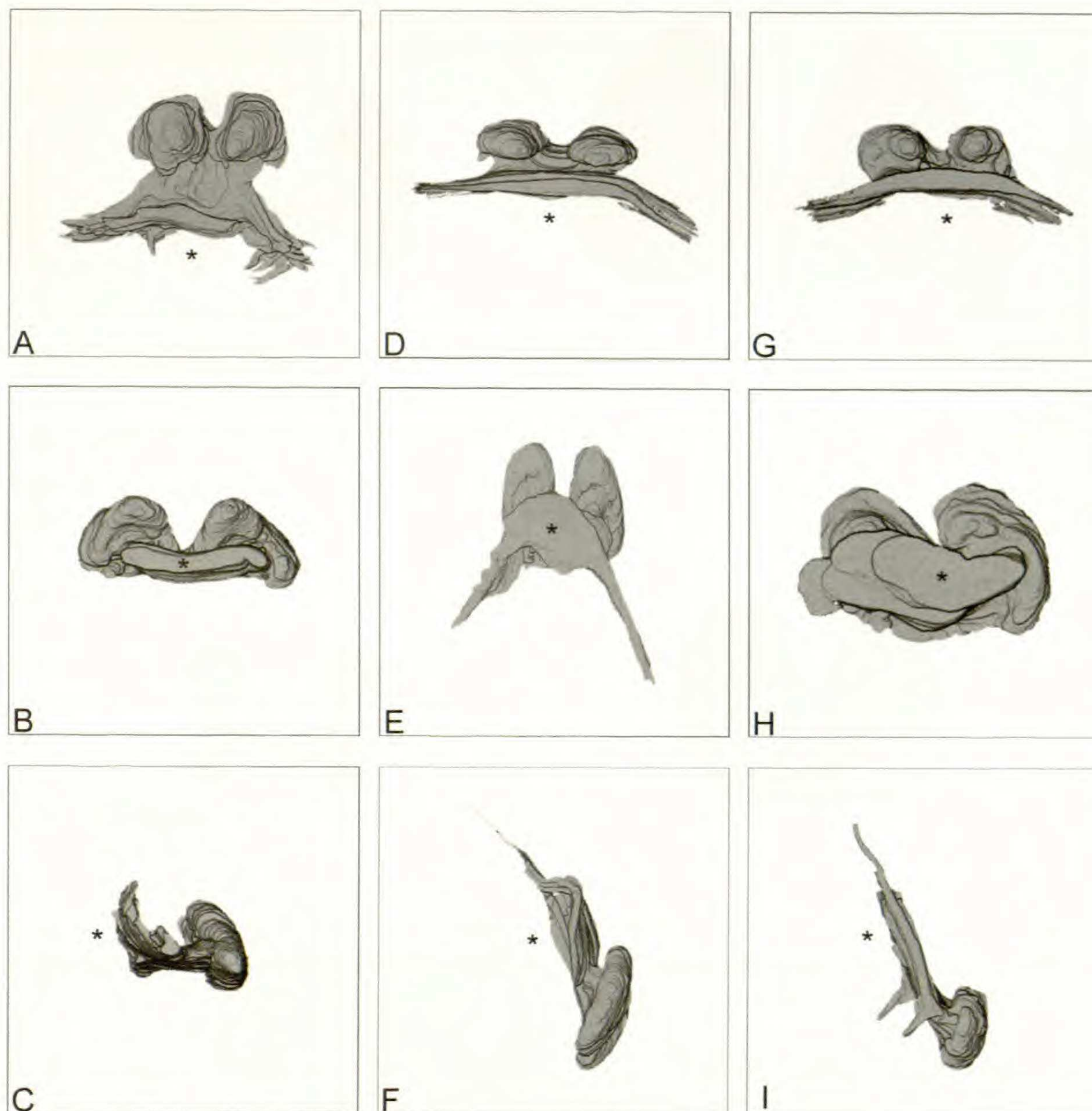


FIG. 8. Reconstructions of three North American *Isoetes* glossopodia. The first column is *I. tennesseensis* (A–C), the second column is *I. virginica* (D–F), and the last column is *I. melanopoda* (G–I). The first row of images is derived from cross sections and the view is from the top (A, D, and, G). The second row of images is derived from paradermal long sections. These views are from an adaxial perspective (B, E, and H). The third row of images are derived from sagittal sections. The views are lateral (C, F, and I). The asterisk references the adaxial sides of each image.

shape (Figs. 4B, 8C) and the ligule is attached slightly below the center of the glossopodium (Figs. 4E, 8C). A well developed medimoles connecting the tongue and glossopodium (Figs. 4B, 8C) is also evident in cross sectional and sagittal views (Figs. 5C, 5D, 8A). In *I. tennesseensis* the glossopodium leans towards the ligule at an acute angle (Figs. 4B, 8C).

The cornua of *I. melanopoda* are ovate in face view (Fig. 8H) and elliptic in side view (Figs. 16B, 8I). The ligule is attached centrally to the adaxial face of

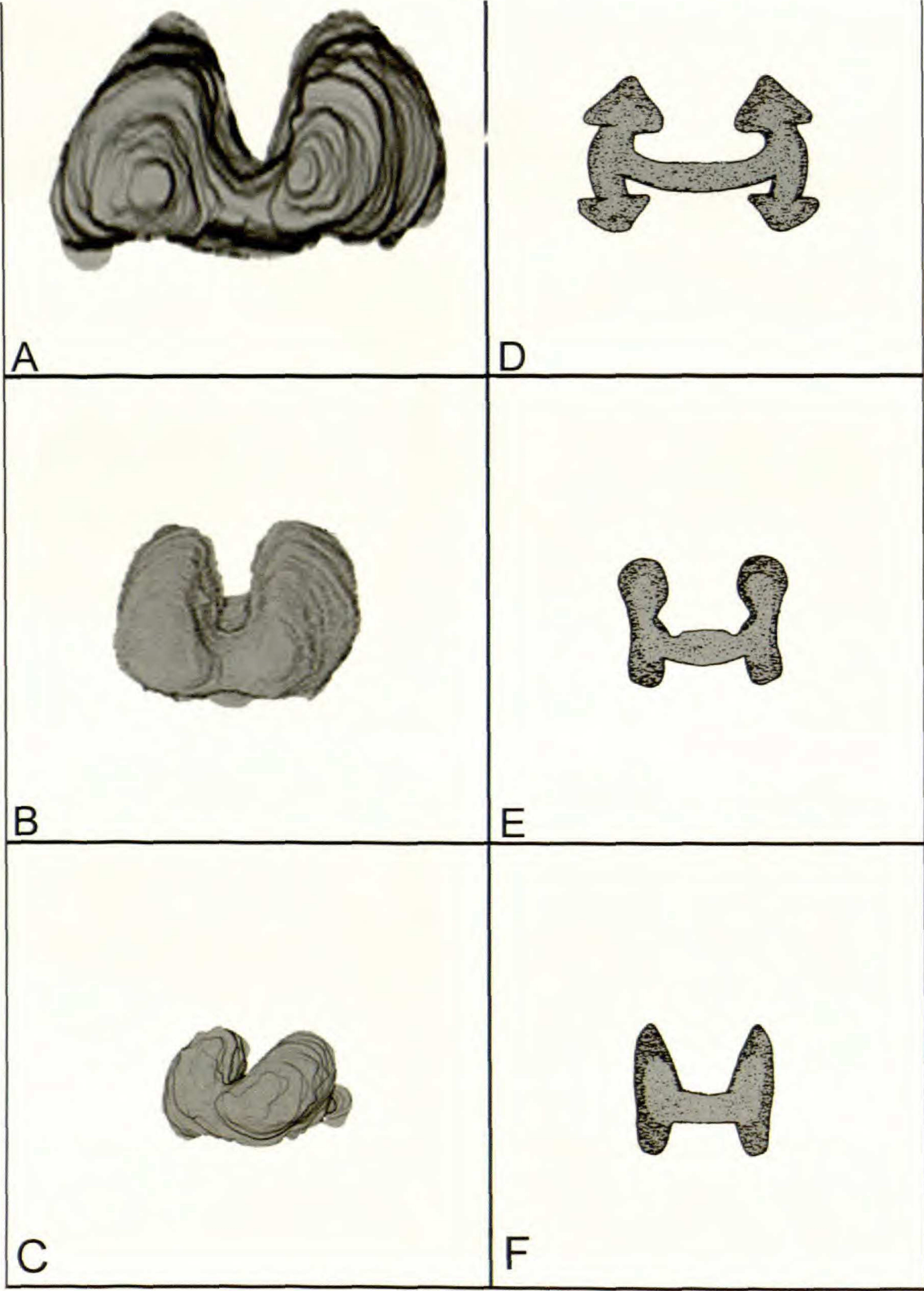


FIG. 9. Reconstructions of the three North American (A–C) and three Indian (D–F) *Isoetes* glossopodia. A. *Isoetes tennesseensis*. B. *I. virginica*. C. *I. melanopoda*. D. *I. coromandelina*. E. *I. rajasthanensis*. F. *I. reticulata*. Figs. A–C are from an abaxial vantage point looking toward the ligules, D–F from an adaxial vantage point; the ligule tongues are not included, but would lie behind the images in A through C. Glossopodia of Indian taxa redrawn and modified from Sharma and Singh, 1984.

the glossopodium by means of a short medimoles (Figs. 6B, 7B, 8G, 8I). In *I. melanopoda*, the upper lobes of the cornua lean slightly away from the ligule (Figs. 1D, 8I) and are angled toward the abaxial side of the leaf.

DISCUSSION

Sharma and Singh (1984) demonstrated variation in size, shape, and complexity in the glossopodia of three Indian species. *Isoetes coromandelina* has the largest, most complex glossopodium with distinctly anchor-shaped cornu lobes (the 'pad-like' structures of Sharma and Singh, 1984) and an extended transverse band (Fig. 9D). *Isoetes rajasthanensis* has a medium-sized glossopodium with globular cornua (Fig. 9E) and *Isoetes reticulata* has the least complex glossopodium of the three, with fusiform cornua (Fig. 9F). The glossopodia of *Isoetes tennesseensis*, *I. virginica*, and *I. melanopoda* are similar in shape. Regardless of this resemblance, there are numerous structural variations among them. They differ in cornu shape, ligule attachment position, size of the medimoles, the angle of departure of the medimoles from the glossopodium, the glossopodium angle, and the maximum height and width of the cornu and glossopodium (Table 2). The glossopodia of the North American species are structurally different from the Indian taxa (Fig. 9 A–F). Based on the descriptions provided by Sharma and Singh (1984), *I. rajasthanensis* (Fig. 9E) most resembles the North American species. The glossopodia of *I. coromandelina* and *I. reticulata* are either too complex or too simple. Due to the small number of images available and their diagrammatic nature however, a more comprehensive study of the Indian species is necessary to fully compare them with the species studied here and with other species.

The reconstructions of the glossopodia created from serial cross, paradermal, and sagittal sections are generally congruent. However, due to the thickness of each section, there is loss of minor detail between reconstructions. For example, the small protuberances and invaginations are often best seen in only one or two sectional planes. It is important for future investigators to include all three sectional planes to insure fine-detail fidelity in reconstructions and illustrations of the glossopodia so that these small differences are not overlooked or interpreted incorrectly. However, these minor variations do not affect the overall appearance of the reconstructions nor does their lack compromise comparisons of overall morphology.

Sharma and Bohra (2002) proposed that the complexity of the glossopodia was somehow linked to the presence of the lacunae. They state that the glossopodium "sends branches toward the four cavities" and the cornua are "arranged in a regular manner into the [air] cavities". Thus, they hypothesize that the lacunae develop prior to the glossopodium. However, many studies suggest that the ligule develops faster than its associated leaf (Smith, 1900a; Bhambie, 1963; Sporne 1966; Sharma and Singh, 1984; Gifford and Foster, 1989) and photographs of longitudinal sections of *Isoetes* corms depict young leaves with well developed glossopodia that lack lacunae (Bierhorst, 1971;

Gifford and Foster, 1989). Even though these studies are inconclusive, they warrant future investigations to determine if Sharma and Bohra's ontogenetic hypothesis is correct.

This work shows that there is variation in glossopodium shape among the three North American taxa. These differences may be due to habit, environmental influences, different ploidy levels or phylogenetic history. Each North American species in this study is from a different habitat. *Isoetes tennesseensis* is an obligate aquatic and as such is rooted in substrates that are considerably less dense than hard-packed terrestrial soils. The lack of substrate pressure on the leaf bases may allow for a looser, less dense packing of leaf bases at the apex of the corm. *Isoetes virginica* is amphibious, and its leaf bases are somewhat compressed by the surrounding substrate resulting in a more compact plant base and shorter, radial leaf base dimensions. *Isoetes melanopoda* is terrestrial, and therefore the entire base of the plant is tightly packed. The substrate undoubtedly exerts pressure on the young leaves, compressing the bases radially, resulting in a very tight, compact plant base and very narrow radial leaf base dimensions. Thus habitat differences could affect the growth patterns of the ligule, effecting changes in the size and shape of the glossopodium. Alternatively, glossopodium variation may reflect chromosome number: each of the North American species examined has a different ploidy level (2 \times , 4 \times , and 8 \times for *Isoetes melanopoda*, *I. virginica*, and *I. tennesseensis* respectively). It is known that plants with higher ploidy levels often have larger cells and organs than plants of lower ploidy (Smith, 1946; Sinnott, 1960). Thus ploidy alone could explain the observed differences in glossopodium size and complexity. Unfortunately, it was not possible to establish a correlation between glossopodia morphology of the Indian species to habit, environmental influences, or different ploidy levels due to the limited information provided by Sharma and Singh (1984). Additional studies on plants of similar chromosome number and varying habitats or different chromosome numbers in a common habitat are required to test these correlations.

If no correlation can be established between form and either chromosome number or habitat preference, then the observed variations may be a function of phylogenetic history. This would be significant because hypotheses of *Isoetes* relationships are complicated due to the simplicity of the plant body, morphological convergence, and reticulate evolution (Taylor and Hickey, 1992). Any new data set therefore would prove valuable. Characters historically used to identify *Isoetes* are habitat, various vegetative features, megaspore ornamentation, and chromosome numbers. Unfortunately, vegetative characters are usually viewed as either too conservative or too variable. For example, the corms, roots, and velum lengths are very similar throughout *Isoetes*, whereas leaf length, ala length, number of leaves per plant, and sporangium size are thought to be dependent on environmental conditions, plant vigor, and age (Kott and Britton, 1985). Leaf texture and color are deemed arbitrary, and non-quantitative; Kott and Britton (1985) argued that they should not be used. An alternative viewpoint on the systematic value of vegetative characters however has been espoused by Hickey (1986a) and Budke

et al. (2005). Furthermore, megaspore ornamentation is more variable (Hickey, 1986b, 1986c) than generally recognized. Since many of the characters used to identify *Isoetes* are not individually conclusive, they should be re-examined and additional morphological characters should be analyzed for taxonomic utility. Some of the character variations noted in this study have the potential of providing not only phylogenetic data but also could serve as an identification aid to various *Isoetes* species in the field. For example, simple hand sectioning of the sporangial region would allow one to analyze relative organ orientations such as those between cornua and ligule or cornua and medimoles; total reconstructs would not be necessary to characterize features such as medimoles and cornua development and orientation.

The glossopodium has been present in lycopsids since the Triassic and is presently found only in *Isoetes* and *Selaginella*. This "relictual" organ must be under some type of selective pressure in order to maintain such a complex form for such a long time. Despite a long history of scientific investigations, there is a great deal we do not understand about this organ and about *Isoetes* itself. The genus continues to be a profitable source of scientific inquiry.

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